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Neonatal Cerebellectomy Alters Ethanol-Induced Sleep Time of Short Sleep but not Long Sleep Mice

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PALMER, M. R., L. OLSON, T. V. DUNWIDDIE, B. J. HOFFER AND Å. SEIGER. *Neonatal cerebellectomy alters ethanol-induced sleep time of short sleep but not long sleep mice.* PHARMACOL BIOCHEM BEHAV **20**(1) 153–159, 1984.—The effects of neonatal cerebellectomy on ethanol-induced sleep times in long sleep (LS) and short sleep (SS) mice were investigated. Cerebellectomy did not alter the ethanol sensitivity of LS animals for loss of righting reflex. In contrast, SS mice became more sensitive to alcohol after cerebellectomy. Even so, large differences were still observed between the alcohol-induced sleep times of cerebellectomized LS and SS mice. The data indicate that, while the cerebellum must have a prominant influence on alcohol sleep time in SS animals, this brain strucutre is not solely responsible for the observed differences in righting reflex sensitivity to ethanol in these two mouse lines. We postulate the existence of noncerebellar central neurons with differential sensitivities to the depressant effects of ethanol in LS and SS mice.

Ethanol sensitivity Cerebellum Cerebellectomy Long sleep mice Short sleep mice

THE hypnotic effects of ethanol have long been under investigation. The development of mouse lines which differ markedly in their soporific response to acute ethanol administration [18] offer a unique opportunity to study the relationship between physiological and behavioral effects of this drug. Sleep time is more than an order of magnitude longer in mice that have long sleep times (LS mice) than in those with short sleep times (SS mice) after a given dose of ethanol [11, 12, 18]. The parental heterogeneous stock of mice (HS mice), derived from a randomized 8-way cross of various inbred mouse strains [18], shows an intermediate sleep time [7]. The time course of blood-ethanol levels are comparable in LS and SS mice after parenteral administration. The blood-ethanol levels are much higher, however, in SS than in LS mice at their respective times of awakening [11,12]. This would suggest that the differential behavioral effects of ethanol in these two mouse lines are due to differential sensitivity of the central nervous system rather than to pharmacokinetic differences. Since sleep time in these studies is defined as the time elapsed between the loss and recovery of the righting reflex, which has been postulated to involve cerebellar function [5,6], cerebellar Purkinje cells in situ have been used as target neurons for examining ethanol effects in our earlier studies.

Previous physiological investigations have implicated the cerebellum as a target brain area in the acute central nervous system effects of systemically administered ethanol [6, 10, 13, 14, 19, 22, 28, 32]. A direct action of ethanol in the cerebellum may be at least partially responsible since the effects of systemic ethanol persist in cerebella which have been surgically isolated from the rest of the brain [8]. Similarly, ethanol alters cerebellar Purkinje cell firing rates when applied locally by micro pressure-ejection or electroosmosis in situ [26, 27, 31] and when perfused over cerebellar brain grafts in oculo [21], cerebellar slices [2], or cerebellar explants in vitro [24]. Correlating with the differential behavioral responsiveness of LS and SS mice to soporific doses of ethanol discussed above, we have found that the spontaneous discharge of cerebellar Purkinje cells is depressed by locally applied ethanol at doses which are 30-fold lower in LS mice than in SS mice [31]. Four lines of evidence from more recent investigations suggest that these previously observed differences in the depressant effects of ethanol on Purkinje cell spontaneous discharge in LS and SS mice may be related to differential soporific actions of this drug. First, the mean ethanol dose needed to elicit equivalent depressions of Purkinje cell activity in HS mice is intermediate between the mean LS and SS values [30]. This agrees well with the intermediate ethanol-induced sleep time in HS animals [7]. Second, no differential effects were seen with local halothane application in LS and SS lines [30]. Again, this agrees with behavioral data showing that halothane-induced sleep time is similar in the LS and SS lines [1]. Third, a high genetic correlation between sleep time and inhibition of cerebellar Purkinje neuron discharge rate in response to acute ethanol administration has been found

amongst the eight inbred mouse strains from which the HS parental stock was derived [29]. Fourth, isolated cerebellar preparations from LS and SS mice, such as in oculo transplants [21] or in vitro slices [2], manifest similar differential P cell sensitivity as is seen in situ. These latter experiments also suggest that the factors which are responsible for determining Purkinje cell sensitivities to ethanol are intrinsic to the cerebellum.

Although Purkinje cells may be one locus for the differential behavioral effects of ethanol in these mouse lines, other neuronal pathways which participate in the righting reflex may also be involved. This could be tested by ablation of the cerebellum. Unfortunately, in adult mammals large cerebellar lesions elicit a significant degree of ataxia and motor incoordination [4]. However, animals receiving neonatal cerebellar lesions possess a significant degree of plasticity in central motor pathways [3, 9, 15, 16, 33], and such animals manifest a testable righting reflex as adults. In this communication, we have studied LS and SS mice which have undergone partial or total neonatal cerebellectomy. We sought to answer two questions. First, to what extent are ethanol-induced changes in "sleep time" dependent on intact cerebellar circuitry and second, if such dependencies exist, are they similar in these two mouse lines?

METHOD

Cerebellectomy

A total of eighteen mouse litters, including long sleep (LS) and short sleep (SS) mice, and ten adult heterogenous stock (HS) mice were used. Pregnant LS and SS mice at the twenty-fourth generation of selection (thirty-third generation of breeding) were supplied by the Institute for Behavioral Genetics of the University of Colorado. Neonatal mice of either sex ranging in postnatal age from 5 to 10 days were cerebellectomized under ether anesthesia. In order to minimize the stress to the mothers, they were removed from their respective litters prior to any handling of the pups; mothers were returned only after the replacement of all cerebellectomized pups in the cage. In addition, the mothers and pups were handled only with surgical gloves, and any excess blood around the wound margin was removed from the pups prior to the return of the mothers. For the surgery, each mouse pup was held with its head bent forward so that the skull and skin overlying the cerebellum was maximally exposed. Using a lancet, a small incision was made in the skin and cartilaginous skull just above and behind the right ear. A sterile No. 12 stainless steel needle (0.7×30 mm), which had been blunted at the tip, was inserted from the side through the opening of the skull. The developing cerebellar anlage was removed by applying gentle suction through the needle. Care was taken to avoid damage to the underlying brainstem. The needle was then removed and one suture was placed in the skin to close the head wound. Often artificial intermittent positive pressure respiration was required for several minutes following the surgery before the mouse pups resumed an acceptable rate of spontaneous respiration.

Cerebellectomized mouse pups, as well as age-paired control mice of either sex, were kept with their original litters until weaning at 21 days of age, and were housed under identical environmental conditions. Postmortem histological analysis showed that the degree of cerebellectomy of animals from any one litter usually ranged from 0% to 100% so that the litter conditions were internally controlled. In addition, those animals which were found to have 100% of their cerebellum remaining acted as internal sham controls. Food pellets and water were provided ad lib and a twelve-hour light-dark cycle was employed. Immediately prior to sleep time determinations, neurological symptoms were evaluated during exploratory and finger avoidance behaviors. For these measurements, animals were taken from their home cage and were individually placed in a fresh "novel" cage. Each animal was observed both while behaving spontaneously and while attempting to avoid the experimenter's hand. Each animal was rated from 1 to 4 as follows:

1=animals showing normal motor and postural behavior.

2=animals which can run and walk relatively normally except that they often move in circles; for any one animal, the circling is always in the same direction. In addition, they tend to stagger while walking, and occasionally appear to show intention tremors. They lean to one side while standing still, and their hind feet are splayed slightly to the sides.

3=animals which can both run and walk although with some difficulty; they often fall, usually to only one side, while often moving in circles. They occasionally stumble forward while walking, sometimes appear to walk on tip toe, and often tremor while walking or standing. Their hind feet are markedly splayed apart, and they lean to one side, often against the wall of the cage while standing still.

4=animals which cannot run and fall from side to side while walking. They have tremors much of the time, and their hind feet are splayed apart. These animals occasionally fall to either side while standing.

Unoperated, sham operated, and cerebellectomized mice of both lines, as well as normal HS mice, were weighed and tested at one to two months of age for sleep time after 4.0 g/kg of IP ethanol (30% v/v in saline). Both experimental and control animals were tested simultaneously. The protocol for estimating sleep time was similar to that used in the selective breeding of the LS and SS mice [17]. Sleep time was measured as the time (in minutes) between loss and recovery of the righting reflex after administration of ethanol. Mice were placed in Plexiglas troughs immediately after loss of the righting reflex. A mouse had to demonstrate the ability to right itself in the trough three consecutive times within a one-minute period after the initial righting, in order for the first recovery to be counted as the end of the sleep time interval. All sleep time measurements were conducted between 8 a.m. and noon on different days. The evaluation of control and cerebellectomized mice from the LS and SS lines for sleep time was randomized with respect to time of morning that testing was initiated on different days, as well as with respect to the day of testing. All behavioral tests on cerebellectomized animals were essentially blind since the percentage cerebellectomy was not determined until the postmortem histological examinations which followed the behavioral tests. Correlation coefficients for sleep time versus percentage cerebellum remaining were calculated by linear least squares regression. Significance of correlation coefficients were determined using t values from the following equation: $t = r\sqrt{n-2}\,\sqrt{1-(r)^2}$.

In our early experiments, comparisons were made between sham operated LS (n=8) and SS (n=7) mice, and age-paired unoperated controls. Since there was no difference between the sleep time of sham operated vs. unoperated mice in either line, and since there was a wide spectrum in the degree of cerebellectomy in any given group (see below), it was decided not to include a sham operated group in the later experiments.

After sleep time measurements, each animal was decapi-

tated under ether anesthesia. The brain was removed and assessed for degree of cerebellectomy by gross morphological examination. The brains of these animals were then fixed in buffered 10% formalin and transferred to a 1 M phosphate buffered solution of 5% sucrose for at least 24 hours prior to sectioning. Brains were sectioned at 6 μm and stained with cresyl violet for routine histological examination.

RESULTS

Approximately 20% of the mice in each line died of respiratory failure within 36 hours after cerebellectomy. Although the percentage cerebellectomy was variable in these animals, all suffered brainstem damage. The remaining 80% survived well until about postnatal day 30 at which time a significant additional mortality due to tonic seizures occurred. This group of animals typically had severe cerebellar damage complicated by varying degrees of collicular and brainstem destruction.

The percentage of cerebellum remaining was determined by gross morphological examination at autopsy (Fig. 1) and was later confirmed by serial reconstructions of cresyl violet stained parasagittal sections (Fig. 2). A wide range of percentages of cerebellum remaining was found for both SS and LS mice (Table 1), including 0% remaining (total cerebellectomy; Figs. 1A and 2B), greater than 75% of the cerebellum remaining (Figs. 1C and 2A) and various intermediate percentages (Figs. 1B, 2C and D). Table 1 illustrates the relationships between percentage of cerebellum remaining, averaged neurological symptoms, and body weight at the time of sleep time testing. The numbers of animals are indicated in parenthesis after the neurological symptom score. There was a strong negative correlation between the percentage of cerebellum remaining and average neurological symptoms for both SS ($r=-0.95, p<0.005$) and LS ($r=-0.88, p<0.005$) lines although the partially cerebellectomized SS mice appeared to be more severely affected than the corresponding LS mice. Similarly, the body weights of the cerebellectomized mice were lower than controls; the totally cerebellectomized LS mice in particular had considerably lower body weights than other groups.

Although cerebellectomy did not appear to alter the ethanol-induced sleep time of LS mice (Fig. 3A, $r=0.28$, NS), SS mice which had received either total or major partial cerebellectomies responded to IP ethanol with considerably prolonged sleep times as compared to controls (Fig. 3B). A striking negative correlation ($r=-0.94, p<0.005$) was found for SS mice between ethanol-induced sleep time and the percentage of cerebellum reamining. Thus, SS mice found to have 0–30% of their cerebellum remaining slept the longest (30–50 min); these values are intermediate between those for control SS mice (15–20 min) and that which was found with normal HS mice (33–152 min). The SS animals which had 50% or more of their cerebellum intact had sleep times which did not differ from controls when given the same IP ethanol dose. The sleep times of those SS animals having 30–50% of their cerebellum remaining were somewhat intermediate. Even though cerebellectomy appeared to lengthen the ethanol sleep time response of SS mice, the longest sleep times from this group of animals were considerably shorter than any of those recorded for cerebellectomized or control LS mice (140–220 min) in this study.

DISCUSSION

The data in this paper, taken together with previous reports (see [23]), suggest that the cerebellum of at least the short sleep mouse plays an important role in determining the duration of ethanol-induced "sleep time." The increased sensitivity of cerebellectomized SS mice to ethanol is in accord with previous work by Northrup [20]. He found that the homozygous "nervous" mutant mice [25], which have lost their cerebellar Purkinje neurons, are more sensitive to the ataxic effects of alcohol than are either heterozygous or normal mice of the same strain. In contrast to the findings with the SS mice, there is little influence of cerebellectomy on the sleep time of mice of the LS line.

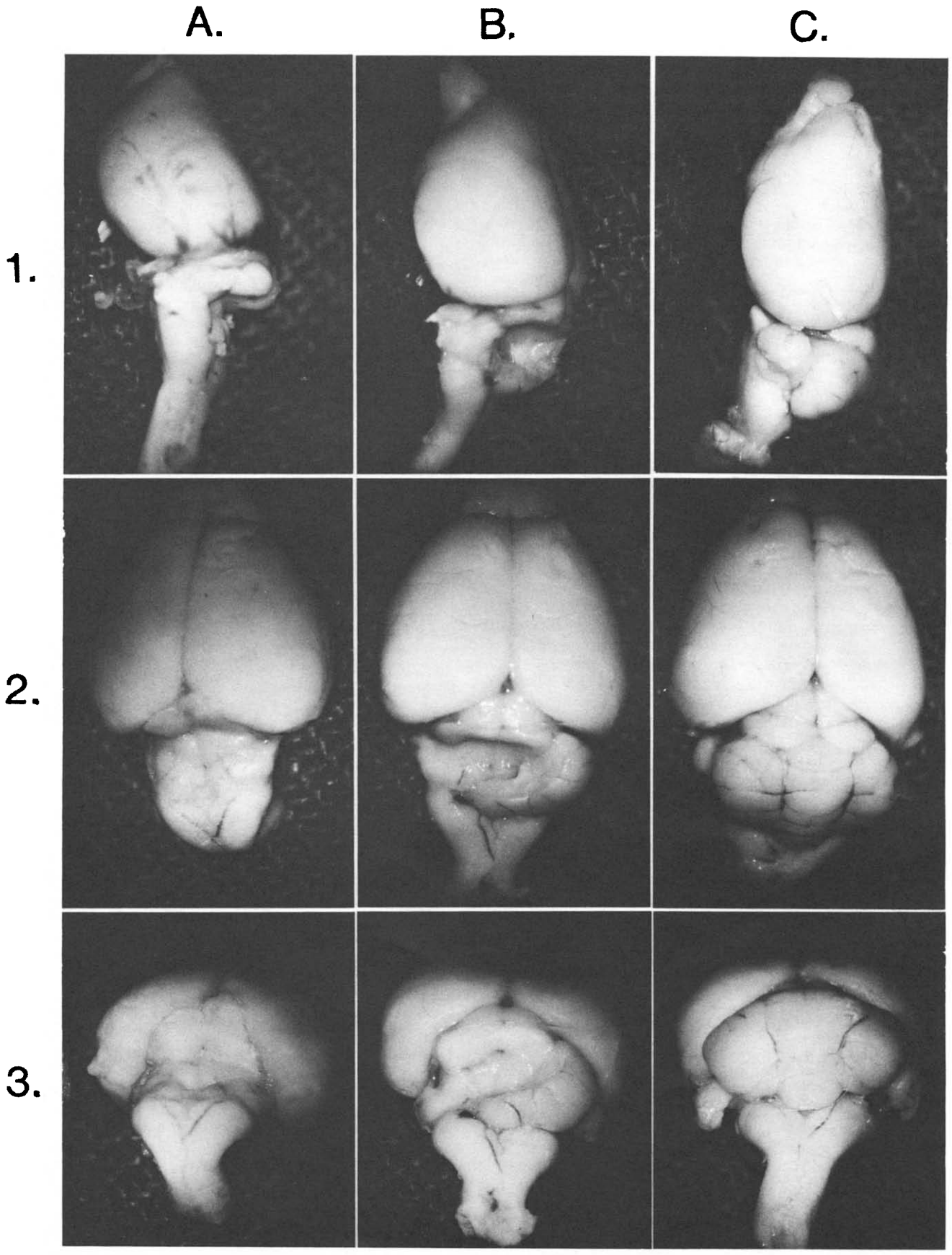
While the differential effect of neonatal cerebellectomy on the two lines of mice is quite apparent, the functional basis for these differences is not. Several hypothesis might be advanced regarding the mechanisms which could underlie the cerebellectomy-induced changes in sleep time of SS but not LS mice.

First, nutritional factors (e.g., change in body weight) may underlie the increased sensitivity to ethanol seen in the SS mice after cerebellectomy. Since the body weights of cerebellectomized mice were much lower than were the controls at the time of sleep time testing, the question arises as to the role of nutrition in the observed differential influence of cerebellectomy. Although this issue cannot be unequivocally answered without "pair feeding" protocols, the fact that the LS mice, which show no influence of cerebellectomy on sleep time, have the larger depression in weight suggests malnutrition is not a significant factor in this differential response.

Second, cerebellectomy may produce a more severe motor impairment in the SS mice, which is reflected in an increased sensitivity to the effects of ethanol. Although the neonatal cerebellectomy did not eliminate the righting reflex response in these animals (as it would in an adult), numerous "neurological symptoms" in terms of motor deficits are still observed in both lines. Indeed, these deficits were significantly more pronounced ($p<0.001$) in the SS mice than in the LS mice as assessed by Wilcox signed ranks test. Thus, it might be argued that SS mice showed the differential effect because they were more affected initially by the lesion. However, this would seem somewhat unlikely because even when the animals are paired on the basis of the severity of the "neurological symptoms," rather than on the extent of the cerebellar lesion, the ethanol sensitivity of SS mice is more affected by the cerebellectomy than that of the LS mice.

Third, the brain regions which underlie the righting reflex in intact LS and SS mice may be different. Previous investigations indicate that a number of neuronal circuitries, including vestibular, propriospinal, and cerebellar, are involved in the righting reflex [5,6]. The relative importance of each of these sites to the behavioral response to alcohol is not known. The previously observed differences in the sensitivities of cerebellar Purkinje neurons to locally applied ethanol has been suggested as a possible explanation for the differences in behavioral responsiveness to ethanol in LS and SS mice [23,31]. However, it is also possible that the selection pressure has resulted in other brain regions becoming the "neuronal substrate" for the righting reflex in the LS mice. Thus, the cerebellum may be of relatively lesser importance to the LS mice for regulating ethanol-induced sleep time than in SS mice. If this was the case, then cerebellectomy might be expected to have little if any effect on ethanol-induced sleep time in the LS line.

Fourth, the brain region(s) which underlie the righting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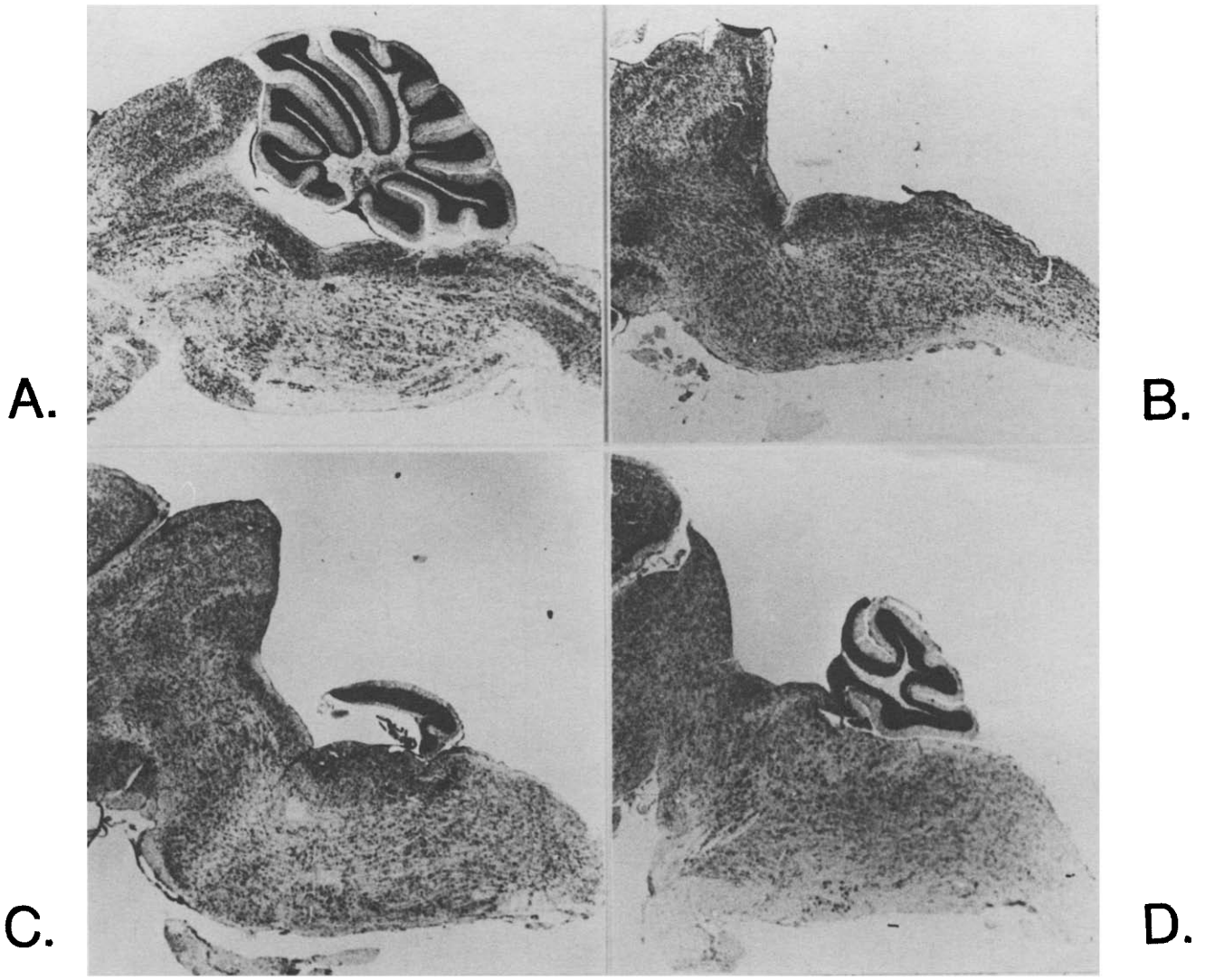

FIG. 2. Photographs of cresyl violet stained, parasagittally sectioned LS and SS brains (A) less than 10% cerebellectomized (>90% cerebellum remaining), (B) totally cerebellectomized (0% cerebellum remaining), (C) 90% cerebellectomized (10% cerebellum remaining), and (D) 70% cerebellectomized (30% cerebellum remaining).

reflex in the cerebellectomized SS mice may not show the same sensitivity to ethanol as does the cerebellum. Although one investigator has found that some locomotor behaviors are more impaired after neonatal hemicerebellectomy, than are lesioned adults [9], we find that neither partial nor total neonatal cerebellectomy eliminates the righting reflex response when tested in mature animals. This is possibly related to the observations from other studies that significant brain reorganization can occur after neonatal cerebellectomy [3, 9, 15, 16, 33] and that cerebellectomized mammals show a considerable recovery of motor function (see [4]). Thus, the plasticity of the neonatal brain appears to permit other brain regions to at least partially replace the role of the cerebellum in this particular function. Perhaps the regions which become important to the righting reflex in the "reorganized" brain of SS mice have ethanol sensitivities which differ from

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FIG. 1. Photographs of mouse brains which are (Column A) totally cerebellectomized (0% cerebellum remaining), (Column B) 70% cerebellectomized (30% cerebellum remaining), and (Column C) less than 10% cerebellectomized (>90% cerebellum remaining). Each brain is shown from side view (row 1), from top view (row 2), and from back view (row 3). Note that the romboid fossa is in the field of view in A_2 but partially obscured by the cerebellar remaining in B_2, and that the caudal opening of the cerebral aqueduct is in full view in A_3 and B_3.

TABLE 1

% Cerebellum Remaining	Short Sleep: Average Neurological Symptoms (1–4)	Short Sleep: Body Weight % of Control	Long Sleep: Average Neurological Symptoms (1–4)	Long Sleep: Body Weight of Control
Control	1.0 (15)	100	1.0 (15)	100
0	3.8 (5)	59.0	3.2 (5)	37.9
0.5–10	4.0 (3)	50.8	3.0 (3)	57.5
10–30	3.2 (5)	63.7	1.7 (3)	83.7
30–50	3.0 (3)	72.6	1.7 (3)	78.7
50–75	1.3 (3)	91.0	1.5 (4)	83.8
>75	1.6 (3)	83.3		

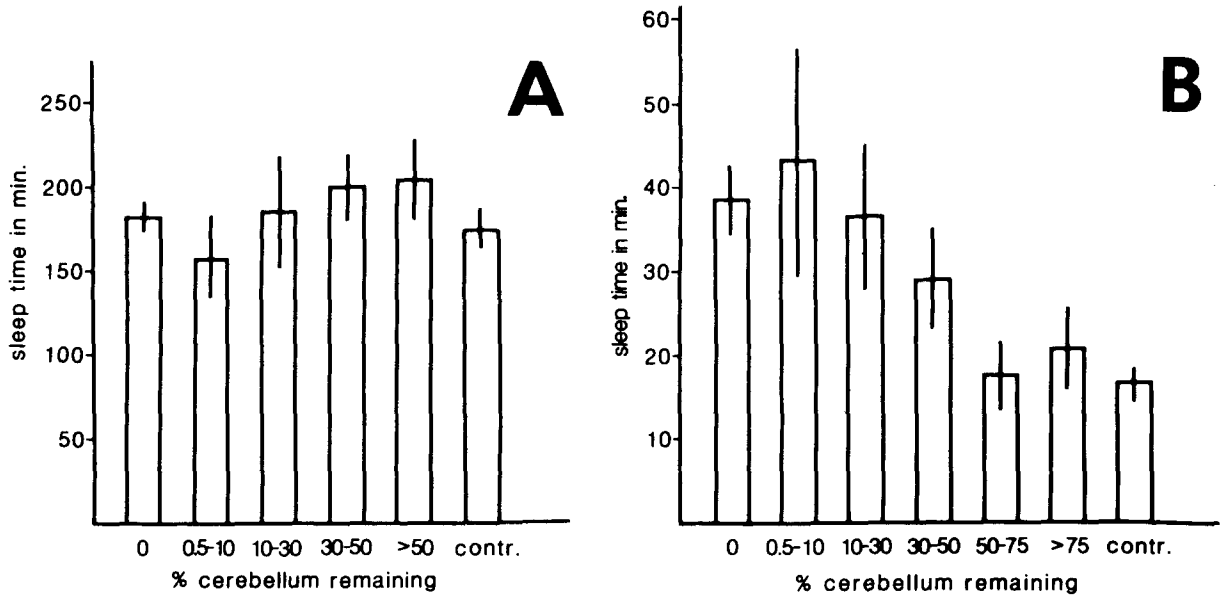

FIG. 3. The effect of cerebellectomy on the ethanol-induced sleep time (±SEM) of LS mice (A) and SS mice (B) which are partially or totally cerebellectomized shortly after birth. The abscissa represents the percentage of cerebellum remaining as assessed by gross morphology at autopsy and confirmed by histological examination at a later date. Cerebellectomy appears to have a significant effect on the sleep times of SS, but not LS mice.

that of the cerebellum. This might explain why cerebellectomy increases the sensitivity of this line to the soporific effects of ethanol. Furthermore, the brain regions which serve the comparable function in the reorganized LS brain may have the same sensitivity as the cerebellum. Thus, cerebellectomy would have little effect on sleep time in these animals.

In summary, it is clear that neonatal cerebellectomy does not eliminate the large sleep time differences between LS and SS mice to IP ethanol. These data suggest that populations of neurons exist in brain regions other than cerebellum which possess differential sensitivities to ethanol between LS and SS mice. However, SS mice do become more sensitive to ethanol after cerebellectomy suggesting possible differences in the brain mechanisms regulating the righting reflex and/or in the plastic reorganization after neonatal brain damage between these two mouse lines. Further investigations of brainstem nuclei which might be involved in the righting reflex in unanesthetized as well as anesthetized animals may prove insightful both for assessing the mechanisms of action of alcohol and for unraveling the linkage between neurophysiological and behavioral effects of ethanol.

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REFERENCES

1. Baker, R., C. Melchior and R. Deitrich. The effect of halothane on mice selectively bred for differential sensitivity to alcohol. *Pharmacol Biochem Behav* **12:** 691–695, 1980.
2. Basile, T., B. Hoffer and T. Dunwiddie. Differential sensitivity of cerebellar Purkinje neurons to ethanol in selectively outbred lines of mice: Maintenance *in vitro* independent of synaptic transmission. *Brain Res* **264:** 69–78, 1983.
3. Castro, A. J. Projections of the superior cerebellar peduncle in rats and the development of new connections in response to neonatal hemicerebellectomy. *J Comp Neurol* **178:** 611–628, 1978.
4. Dow, R. S. and G. Moruzzi. *The Physiology and Pathology of the Cerebellum*. Minneapolis: University of Minnesota Press, 1958.
5. Eccles, J., M. Ito and J. Szentagothai. *The Cerebellum as a Neuronal Machine*. New York: Springer-Verlag, 1967.
6. Eidelberg, E., M. Bond and A. Kelter. Effects of alcohol on cerebellar and vestibular neurons. *Arch Int Pharmacodyn Ther* **192:** 213–219, 1971.
7. Erwin, V., W. Heston, G. McClearn and R. Deitrich. Effect of hypnotics on mice genetically selected for sensitivity to ethanol. *Pharmacol Biochem Behav* **4:** 679–683, 1976.
8. Forney, E. and W. R. Klemm. Effect of ethanol on impulse activity in isolated cerebellum. *Res Commun Chem Pathol Pharmacol* **15:** 801–804, 1976.
9. Gramsbergen, A. and J. Ijkema-Paassen. CNS plasticity after hemicerebellectomy in the young rat. *Neurosci Lett* **33:** 129–134, 1982.
10. Grupp, L. A. and E. Perlanski. Ethanol induced changes in the spontaneous activity of single units in the hippocampus of the awake rat: A dose response study. *Neuropharmacology* **18:** 13–70, 1979.
11. Heston, W., S. Anderson, V. Erwin and G. McClearn. A comparison of the actions of various hypnotics in mice selectively bred for sensitivity to ethanol. *Behav Genet* **3:** 402–403, 1973.
12. Heston, W., V. Erwin, S. Anderson and H. Robbins. A comparison of the effects of alcohol on mice selectively bred for differences in ethanol sleep time. *Life Sci* **14:** 356–370, 1974.
13. Kalant, H. Ethanol and the nervous sytem: Experimental neurophysiological aspects. *Int J Neurol* **9:** 111–124, 1974.
14. Klemm, W. R. and R. E. Stevens, III. Alcohol effects on EEG and multiple unit activity in various brain regions of rats. *Brain Res* **70:** 351–368, 1974.
15. Leong, S. K. A qualitative electron microscopic study of the corticopontine projections after neonatal cerebellar hemispherectomy. *Brain Res* **194:** 299–310, 1980.
16. Lim, K. H. and S. K. Leong. Aberrant bilateral projections from the dentate and interposed nuclei in albino rats after neonatal lesions. *Brain Res* **96:** 306–309, 1975.
17. McClearn, G. E. and R. Kakihana. Selective breeding for ethanol sensitivity: short-sleep and long-sleep mice. In: *Development of Animal Models as Pharmacogenetic Tools*, Research Monograph No. 6, edited by G. E. McClearn, R. A. Dietrich and V. G. Erwin. Rockville, MD: National Institute on Alcohol Abuse and Alcoholism, 1973, pp. 281–315.
18. McClearn, G. E., J. R. Wilson and W. Meredith. The use of isogenic and heterogenic mouse stocks in behavioral research. In: *Contributions to Behavior-Genetic Analysis: The Mouse as a Prototype*, edited by G. Lindzey and D. O. Thiessen. New York: Appleton-Century-Crofts, 1970, pp. 3–22.
19. Mitra, J. Differential effects of ethanol on unit activity in cerebellum and other brain areas in the rat. *Soc Neurosci Abstr* **3:** 298, 1977.
20. Northrup, L. R. Additive effects of ethanol and Purkinje cell loss in the production of ataxia in mice. *Psychopharmacology (Berlin)* **48:** 189–192, 1976.
21. Palmer, M. R., S. Sorensen, R. Freedman, L. Olson, B. J. Hoffer and Å. Seiger. Differential ethanol sensitivity of intraocular cerebellar grafts in long-sleep and short-sleep mice. *J Pharmacol Exp Ther* **222:** 480–487, 1982.
22. Rogers, J., G. R. Siggins, J. A. Schulman and F. E. Bloom. Physiological correlates of ethanol intoxication tolerance and dependence in rat cerebellar Purkinje cells. *Brain Res* **916:** 183–198, 1980.
23. Seiger, Å., S. M. Sorensen and M. R. Palmer. Cerebellar role in the differential ethanol sensitivity of long-sleep and short-sleep mice. *Pharmacol Biochem Behav* **18:** Suppl 1, 465–499, 1983.
24. Seil, F. J., A. L. Leiman, M. M. Herman and R. A. Fisk. Direct effects of ethanol on central nervous system cultures: An electrophysiological and morphological study. *Exp Neurol* **55:** 390–404, 1977.
25. Sidman, R. L. and M. C. Green. "Nervous," a new mutant mouse with cerebellar disease. In: *Les Mutants Pathologiques Chez l'Animal*, edited by M. Soboundy. Paris Centre National de la Recherche Scientifique, 1970.
26. Siggins, G. R. and F. E. Bloom. Alcohol-realted electrophysiology. *Pharmacol Biochem Behav* **13:** Suppl 1, 203–211, 1980.
27. Siggins, G. R. and E. French. Central neurons are depressed by iontophoretic and micropressure application of ethanol and tetrahydropapaveraline. *Drug Alcohol Depend* **4:** 239–243, 1979.
28. Sinclair, J. G. and G. F. Lo. Acute tolerance to ethanol on the release of acetylcholine from the cat cerebral cortex. *Can J Physiol Pharmacol* **56:** 668–670, 1978.
29. Spuhler, K., B. Hoffer, N. Weiner and M. Palmer. Evidence for genetic correlation of hypnotic effects and cerebellar Purkinje neuron depression in response to ethanol in mice. *Pharmacol Biochem Behav* **17:** 569–578, 1982.
30. Sorensen, S., T. Dunwiddie, G. McClearn, R. Freedman and B. Hoffer. Ethanol-induced depressions in cerebellar and hippocampal neurons of mice selectively bred for differences in ethanol sensitivity: An electrophysiological study. *Pharmacol Biochem Behav* **14:** 227–234, 1981.
31. Sorensen, S., M. Palmer, T. Dunwiddie and B. Hoffer. Electrophysiological correlates of ethanol-induced sedation in differentially sensitive lines of mice. *Science* **210:** 1143–1145, 1980.
32. Wayner, M. J., T. Ono and D. Nolley. Effects of ethyl alcohol on central neurons. *Pharmacol Biochem Behav* **3:** 499–506, 1975.
33. Yamamoto, T., S. Kawaguchi and A. Samejima. Electrophysiological studies on plasticity of cerebellothalamic neurons in rats following neonatal hemicerebellectomy. *Jpn J Physiol* **31:** 217–224, 1981.